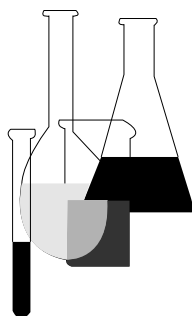




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# Health Effects Test Guidelines

## OPPTS 870.7200 Companion Animal Safety



## INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

**Final Guideline Release:** This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512-0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

## **OPPTS 870.7200 Companion animal safety.**

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP 81–6 Domestic Animal Safety Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982.

(b) **Purpose.** (1) Companion animal safety studies are intended to demonstrate that pesticide formulations for the treatment of external pests on domestic animals have an adequate margin of safety if the products are misused (overused). Data from companion animal safety studies also serve as a basis for product labeling. This guideline is intended to promote uniform review of data and to assure consistency and fairness in the requirements for these studies. Although not a toxicity study of the type required for pesticide registration, a companion animal safety study is most comparable to an acute dermal toxicity study.

(2) This guideline also serves the purpose of providing harmonization between the Environmental Protection Agency and the Center for Veterinary Medicine in the Food and Drug Administration (FDA), which is also responsible for conducting target animal safety studies.

(3) This guideline is limited to products for use on dogs and cats due to the high volume use of products in these two species. The guidance is based on professional experience, documentation in the scientific literature, and policy and procedures of other agencies involved in regulatory veterinary medicine. (see Target Animal Safety Guidelines for New Animal Drugs, under paragraph (i)(3) of this guideline.)

(4) The guideline addresses data requirements for the safety assessment of products applied directly to animals. Products used to treat external pests on domestic animals include, but are not limited to collars, sprays, dips, shampoos, and spot treatments. Due to differences in methods of application, specific testing procedures for individual products are dependent on label claims. Labeled uses also impact on the duration of treatment and on the age and species of test animal used in a companion animal safety study.

(5) The studies conducted to satisfy this guideline should not be mistaken for toxicity studies, as their intent is not to establish a no-observed-effect-level (NOEL), but to provide assurance that an adequate margin of safety exists.

(6) Studies conducted to satisfy companion animal safety guidelines should be conducted in compliance with 40 CFR part 792 and 40 CFR

160 (Good Laboratory Practice Standards) and a statement of compliance should be contained within the final report.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR part 792 apply to this test guideline. The following definitions also apply to this test guideline.

*Acute dermal toxicity* is the adverse effect occurring during or following a dermal exposure to a single dose of a test substance or to repeat applications to achieve dose. For purposes of companion animal safety studies, the test substance is always the final formulation of a pesticide product.

*Adverse effect* is an undesirable effect reflected in the animal by alterations in structure, function, or behavior.

*Companion animal* will be limited to dogs and cats for the purpose of this guideline.

*Companion animal safety study* is a study conducted for the purpose of establishing an adequate margin of safety and not a NOEL of toxicity.

*Dosage* is a term comprising the dose, its frequency, and the duration of dosing.

*Dose* is the amount of test substance administered and is expressed in weight of test substance per unit weight of the test animal (e.g. milligrams per kilogram).

*Margin of safety* is the difference between the effective dose (recommended dose) and the toxic dose. The companion animal safety study guideline does not require the determination of a toxic dose but rather the establishment of an adequate margin of safety.

*Max/tox* is a product containing multiple toxicants at the maximum levels which would be anticipated in formulated topical products.

*Vehicle* is the end-use product formulation, i.e. the inert ingredients without the active ingredients.

(d) **Principle of the test method.** (1) The design of a companion animal safety study should reflect the product label, i.e. the method of administration, species and age group, frequency of application, etc., used in the study should be identical to that of the end-use product. The test formulation should be applied to several groups of experimental animals at the label recommended dose and multiples of this dose (3X and 5X the recommended dose).

(2) If the product label states that a treatment can be repeated, the companion animal safety study should also include a repeat treatment.

(3) Observations and measurements of possible treatment-related effects should be reported for 14 days posttreatment. Animals that die or are sacrificed in a moribund state should be subjected to a necropsy in an attempt to arrive at the cause of death. Routine sacrifice or necropsy is not required for surviving animals.

(e) **Substance to be tested.** (1) The end-use product should be tested at the recommended dose (1X). For exaggerated doses (3X and 5X the recommended dose), products specifically prepared for this type of study that contain higher concentrations (3X and 5X) of the active ingredient are preferred. See additional discussion under paragraph (g)(3)(iii) of this guideline.

(2) Because of the practice of combining several pesticides in one product, a procedure has been proposed whereby maximum concentrations of multiple active ingredients have been used to determine the margin of safety of end-use products. This practice has been referred to as the max/tox procedure.

(f) **Limit test.** If a test at one dose level of at least 5X the recommended dose, using the procedures described for the study, produces no evidence of treatment-related toxicity, a full study using a minimum of three dose levels may not be necessary.

(g) **Test procedures—(1) Animal selection—(i) Species.** The species recommended for treatment on the product label should be included in the study. Studies should be performed on healthy dogs and cats representative of the classes of dogs and cats (size, weight range, sex, or age) for which the product is intended.

(ii) **Age.** The age of animals in the study is dependant upon label claims. If only adults (6 months or older) are the targeted population of animals to receive treatment, adults only will suffice. However, if a product is registered for use on pediatric animals (i.e. puppies and kittens), the label should state a minimum age for this group, for example, “Do not use on puppies (kittens) less than eight weeks of age”. Consequently, the product should be tested in 8 week-old animals in the companion animal safety study.

(iii) **Sex.** (A) Equal numbers of animals of each sex are recommended for each dosage level.

(B) Females should be nonpregnant.

(iv) **Numbers.** At least six animals per sex should be used at each dosage level.

(v) **Pretreatment.** Animals should be vaccinated, dewormed, and acclimated for 2 weeks prior to the initiation of the study. They should be examined by a veterinarian and their suitability should be ascertained prior

to inclusion in the study. Animals should be free of infectious diseases which could complicate the interpretation of the study results.

(2) **Control group.** A concurrent vehicle control group is recommended. Negative (untreated) controls may occasionally be employed to determine whether adverse effects are due to the inert ingredients in a formulation.

(3) **Dosing**—(i) **Dose levels.** The dose levels of the end-use product to be tested should include control (0X), 1X, 3X, and 5X the recommended dose. The targeted adequate margin of safety is 5X. Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs). The route of administration should be the proposed label route.

(ii) **Vehicle.** The vehicle control should be administered at a 5X level. The vehicle should contain the inert ingredients at the maximum levels that would appear in the 5X formulation.

(iii) **Methods of achieving exaggerated doses.** It is preferred that formulations be revised to include exaggerated amounts of the active ingredient. However, if this cannot be achieved because of volume constraints or the physical properties of the ingredients (active or inert), multiple treatments at frequent intervals during the same dosing period will be acceptable.

(A) Spray, dip or shampoo formulations may be applied at the recommended dose at hourly intervals to achieve an exaggerated dose, i.e. three times for a 3X dose and five times for a 5X dose. Other methods to achieve exaggerated doses will be considered on a case-by-case basis.

(B) Multiple collars may be worn simultaneously by the experimental animals to achieve an exaggerated dose.

(4) **Observation period.** The observation period should be at least 14 days following the last treatment. The time at which clinical signs of toxicity appear and disappear should be recorded. The duration of the observation period should not be rigid, but should be determined by the toxic reactions, the rate of onset and the length of the recovery period.

(5) **Administration.** (i) The route of administration for these products should be by the topical or dermal route. The product should be applied in accordance with the label directions. No clipping of the hair or preparation of the skin is required unless such directions appear on the label.

(ii) If a single dose is not possible, multiple treatments at frequent intervals during the same dosing period will be acceptable.

(iii) If the product label recommends repeat treatments, multiple treatments should be included in a companion animal safety study. When re-

peat treatments are required, products will be handled on a case-by-case basis with the retreatment interval being driven by label claims and instructions for use. For fleas collars, a one month duration of study may be sufficient for well-characterized products. Several months may be required for new chemical entities or collars containing multiple active ingredients. Exceptions would include:

(A) Single ingredient diluted products intended for use on dogs only, where chronic studies in the intended species exist with the technical product.

(B) Products with retreatment intervals of 30 days or more.

(C) Products with retreatment intervals of 14 to 30 days which have no observed toxicity following exposure to a 5X dose level.

It is advisable that when complex issues arise, protocols be submitted in advance for review and comment.

**(6) Observations of animals.** (i) Careful clinical observations should be conducted at hourly intervals on the day of treatment for at least 4 h after the last treatment and twice daily thereafter for the duration of the observation period.

(ii) If adverse reactions are observed, the observation period on the day of treatment should be extended to a time at which no further adverse reactions are observed.

(iii) Observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, respiratory system, circulatory system, autonomic and central nervous system, somatomotor activity, and behavior pattern. Particular attention should be directed to observations of central nervous system signs (seizures, tremors, salivation), vomiting and diarrhea.

(iv) The following provides a more complete list of possible observations.

(A) Ocular: Corneal opacities, nystagmus, pupillary changes, blepharospasm, blindness, iritis, chemosis, photophobia, congestion, blanching, discharge, and conjunctivitis.

(B) Equilibrium: Unsteadiness (walking or standing), incoordination, ataxia or paresis, and abnormal reflexes.

(C) Muscular disturbances: Localized or generalized tremors, lip drooping and/or salivation, paralysis, atony, and atrophy.

(D) Behavior (mental attitude): Anxious, apprehensive, circling, comatose, depressed, sedated, restless, panting, convulsions, and aggression.

(E) Integument: Alopecia, haircoat condition, status of hydration, pruritus, and erythema.

(F) Gastrointestinal: Consistency of stools, propulsive diarrhea, hyperactive gut, abdominal muscle tenseness, and vomiting.

(G) Cardiovascular: Heart rate, heart rhythm, and color of mucous membranes.

(H) Appetite/general health: Body weight, feed consumption, and water consumption.

(I) Respiratory: Respirations/minute, dyspnea, respiratory sounds, color of mucous membranes, nasal discharge, apnea.

(v) Individual body weights should be measured twice during the acclimation period, with the second measurement being taken immediately prior to the initiation of treatment, and on day-7 and day-14 of the observation period.

(vi) Individual food consumption should be measured on a daily basis.

(vii) The time of death should be reported for animals dying or sacrificed moribund. Gross necropsies should be conducted to determine the cause of death; abnormal organs/tissues should be examined histologically.

(viii) Clinical pathology should be assessed prior to treatment, 24 h posttreatment and, if altered, on day-7. Clinical pathology examinations are required to determine the possibility of a treatment-related effect on hematology and clinical chemistry parameters, regardless of whether or not clinical signs of toxicity are evident. The following examinations should be made:

(A) Hematology; Hemoglobin, mean corpuscular volume, hematocrit, mean corpuscular hemoglobin, red blood cell count, mean corpuscular hemoglobin concentration, white blood cell count, white blood cell differential count, prothrombin time, and activated partial thromboplastin time.

(B) Clinical chemistry. Glucose, creatinine, sodium, chloride, potassium, phosphorus, total protein, albumin, globulin, calcium, blood urea nitrogen, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total and direct bilirubin.

(C) If the compound is a known cholinesterase inhibitor, plasma, and red cell cholinesterase should be examined pretreatment, and at 6 to 8, 24, and 48 h, posttreatment.

(h) **Data and reporting**—(1) **Treatment of results.** (i) Data should be summarized in tabular form, showing, for each test group:

(A) The number, age and sex of the animals at the start of the study. Results from adult and pediatric animals should be separated.

(B) The number of animals displaying clinical signs of toxicity, a description of the observations and the time of onset.

(C) Individual and group mean values for body weight, food consumption, and clinical pathology measurements.

(D) Time and cause of death, if known, for animals which died or were sacrificed in a moribund condition.

(ii) All observed results should be evaluated by an appropriate statistical method that was selected during the design of the study.

(2) **Evaluation of study results.** The evaluation should include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioral abnormalities, clinical abnormalities, body weight changes, effects on mortality and cause of death, identified target organ, and any other general or specific acute toxic effect.

(3) **Test report.** In addition to information required under 40 CFR part 792, subpart J, and 40 CFR part 160, subpart J, the test report summary should include the following information.

(i) Toxic response and other effects data by sex and dose.

(ii) Species and breed used.

(iii) Individual animal data for the following:

(A) Time of death during the study or whether animals survived to termination.

(B) Time of observation of each abnormal sign and its subsequent course.

(C) Food consumption.

(D) Body weight data.

(E) Hematology tests employed and the results.

(F) Clinical chemistry tests employed and the results.

(G) Necropsy findings on animals that died or were sacrificed in a moribund condition.

(H) Detailed description of histological findings on animals that died or were sacrificed in a moribund condition.

(I) Statistical treatment of results.

(J) Other observations and/or findings.

(i) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Hoskins, J.D. *Veterinary Pediatrics. Dogs and Cats From Birth to Six Months*. Saunders, Philadelphia (1990).

(2) Jones, B.D. *Liver and Pancreatic Disease*. Presented to D.C. Academy of Veterinary Medicine, Fairfax, VA, June 9, 1994.

(3) Target Animal Safety Guidelines for New Animal Drugs. Prepared by the Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, June 1989.